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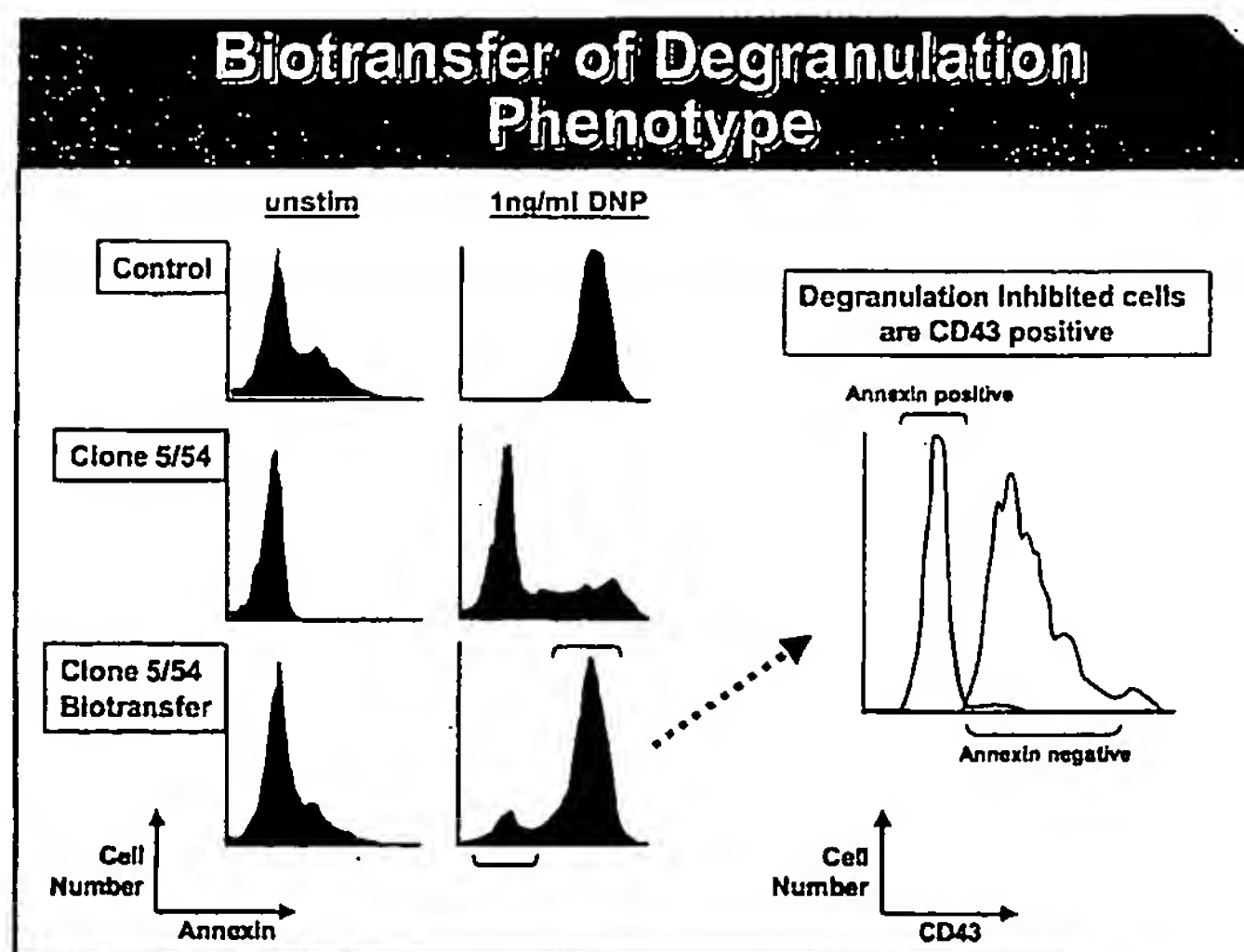
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[Continued on next page]

(54) Title: CD43:MODULATORS OF MAST CELL DEGRANULATION



(57) Abstract: The present invention relates to regulation of IgE-receptor-mediated mast cell degranulation. More particularly, the present invention is directed to nucleic acids encoding CD43 (also called leukosialin or leukocyte large sialoglycoprotein), which is involved in modulation of IgE-receptor-mediated mast cell degranulation. The invention further relates to methods for identifying and using agents, including small molecule chemical compositions, antibodies, siRNA, antisense nucleic acids, and ribozymes, that modulate IgE-receptor-mediated mast cell degranulation via modulation of CD43 and CD43-related signal transduction; as well as to the use of expression profiles and compositions in diagnosis and therapy related to diseases such as allergies and asthma.

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CD43: MODULATORS OF MAST CELL DEGRANULATION

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to USSN 60/296,801, filed June 7,
5 2001, herein incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

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FIELD OF THE INVENTION

The present invention relates to regulation of IgE-receptor-mediated mast cell
degranulation. More particularly, the present invention is directed to nucleic acids encoding
CD43 (also called leukosialin or leukocyte large sialoglycoprotein), which is involved in
15 modulation of IgE-receptor-mediated mast cell degranulation. The invention further relates
to methods for identifying and using agents, including small molecule chemical
compositions, antibodies, siRNA, antisense nucleic acids, and ribozymes, that modulate IgE-
receptor-mediated mast cell degranulation via modulation of CD43 and CD43-related signal
transduction; as well as to the use of expression profiles and compositions in diagnosis and
20 therapy related to diseases such as allergies and asthma.

BACKGROUND OF THE INVENTION

Mast cell degranulation plays a critical role in immediate hypersensitivity
diseases, such as allergies and asthma. Identifying membrane proteins, their ligands, and
25 downstream signal transduction pathways is important for developing therapeutic regents to
treat allergies and asthma.

SUMMARY OF THE INVENTION

The present invention therefore provides nucleic acids encoding CD43, which
30 is involved in modulation of mast cell degranulation and immediate hypersensitivity-type
inflammatory reactions (*see, e.g.*, Paul, Fundamental Immunology (3rd ed., 1993)). The
invention therefore provides methods of screening for compounds, e.g., small molecules,

antibodies, siRNA, antisense molecules, and ribozyme, that are capable of modulating mast cell degranulation and immediate hypersensitivity-type inflammatory reactions, e.g., inhibition of such reactions for treatment of asthma and allergies. Therapeutic and diagnostic methods and reagents are also provided.

5 In one aspect of the invention, nucleic acids encoding CD43 membrane receptors are provided. In another aspect, the present invention provides nucleic acids, such as probes, siRNA, antisense oligonucleotides, and ribozymes, that hybridize to a gene encoding CD43. In another aspect, the invention provides expression vectors and host cells comprising CD43-encoding nucleic acids. In another aspect, the present invention provides
10 CD43 protein, and antibodies thereto.

In another aspect, the present invention provides a method for identifying a compound that modulates IgE-receptor mediated mast cell degranulation and immediate hypersensitivity-type inflammatory reactions, the method comprising the steps of: (i) contacting the compound with a CD43 polypeptide; and (ii) determining the functional effect
15 of the compound upon the CD43 polypeptide.

In one embodiment, the functional effect is a physical effect or a chemical effect. In one embodiment, the polypeptide is expressed in a eukaryotic host cell. In another embodiment, the functional effect is determined by measuring receptor or signal transduction activity, e.g., increases in intracellular calcium or other signaling compounds. In another
20 embodiment, the functional effect is determined by measuring degranulation, e.g., annexin, MAP kinase activation, and tyrosine phosphorylation

In another aspect, the present invention provides a method of modulating mast cell degranulation and immediate hypersensitivity-type inflammatory reactions in a subject, the method comprising the step of contacting the subject with an therapeutically effective
25 amount of a compound identified using the methods described herein.

In another aspect, the present invention provides a method of detecting the presence of CD43 nucleic acids and polypeptides in human tissue, the method comprising the steps of: (i) isolating a biological sample; (ii) contacting the biological sample with a CD43-specific reagent that selectively associates with CD43; and, (iii) detecting the level of CD43-
30 specific reagent that selectively associates with the sample.

In one embodiment, the human CD43-specific reagent is selected from the group consisting of: human CD43-specific antibodies, human CD43 specific oligonucleotide primers, and human CD43-nucleic acid probes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides exemplary nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of wild type human CD43.

Figure 2 shows biotransfer of degranulation phenotype with a cDNA encoding
5 CD43.

Figure 3 shows that CD3 overexpression inhibits IgE-induced degranulation.

Figure 4 shows that CD43 overexpression inhibits anti-IgE-induced tyrosine phosphorylation and MAP kinase activation.

10 DETAILED DESCRIPTION OF THE INVENTION

INTRODUCTION

For the first time, a protein called CD43 has been identified as a membrane receptor involved in modulation of mast cell degranulation and immediate hypersensitivity-type inflammatory reactions (*see, e.g., Pallant et al., Proc. Nat'l Acad. Sci USA 86:2819-2823*
15 (1989); Shelley *et al., Proc. Nat'l Acad. Sci. USA 86:2819-2823* (1989); Shelley *et al., Biochem. J.* 270:569-576 (1990); Kudo & Fukuda, *J. Biol. Chem.* 266:8483-8489 (1991); Rogaev & Keryanov, *Hum. Mol. Genet.* 1:657 (1992)). CD43 was identified from a functional genetic screen that selects for cells with inhibited mast cell degranulation phenotype. An exemplary amino acid and nucleotide sequence of human wild type CD43 is
20 shown in Figure 1. CD43 is an abundantly and broadly expressed leukocyte cell surface mucin, also called leukosialin and sialomucin.

CD43 without wishing to be bound by theory, CD43 appears to act as an anti-adhesion molecule (by steric obstruction or repulsion) and also as a pro-adhesion molecule (ligands include ICAM-1, E-selectin, and MHC class I). CD43 is a cell surface molecule that
25 is phosphorylated by PKC, and associates with CD3 under mild detergent conditions. CD43 crosslinking engages signaling cascades that include fyn, lyn, and syk. CD43 signaling also inhibits apoptosis. CD43 is also involved in cytoskeletal regulation, as the cytoplasmic domain binds ERMs.

CD43 therefore represent a drug target for compounds that inhibit mast cell
30 degranulation and immediate hypersensitivity-type inflammatory reactions. Agents identified in these assays, including small molecule chemical compositions, antibodies, siRNA, antisense nucleic acids, and ribozymes, that inhibit mast cell degranulation and immediate hypersensitivity-type inflammatory reactions via modulation of CD43 and CD43 related signal transduction, can be used to treat diseases such as asthma and allergies. Such

modulators are useful for treating allergies such as contact dermatitis (e.g., caused by poison ivy, rubber, nickel, and industrial metal exposure), allergies caused by drugs such as penicillin, food allergies, stinging insect allergies, latex allergies, allergic rhinitis caused by airborne antigens such as tree, ragweed and grass pollens, mold spores, animal dander, and dust mites, atopic dermatitis, and allergic reactions such as anaphylaxis. Such modulators are also useful for treating both allergic and idiosyncratic asthma.

DEFINITIONS

By "disorder or disease associated with mast cell degranulation" or "disorder of disease associated with immediate type hypersensitivity inflammatory reaction" herein is meant a disease state which is marked by either an excess of mast cell degranulation and inflammation. Such disorders associated with increased mast cell degranulation include, but are not limited to, asthma and allergies.

The terms "CD43" or a nucleic acid encoding "CD43" refer to nucleic acids and polypeptide polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 60% amino acid sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of over a region of at least about 25, 50, 100, 200, 500, 1000, or more amino acids, to an amino acid sequence encoded by an CD43 nucleic acid or amino acid sequence of an CD43 protein (*see, e.g.,* Figure 1); (2) bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of an CD43 protein, and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to an anti-sense strand corresponding to a nucleic acid sequence encoding an CD43 protein, and conservatively modified variants thereof; (4) have a nucleic acid sequence that has greater than about 95%, preferably greater than about 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, or more nucleotides, to an CD43 nucleic acid. A polynucleotide or polypeptide sequence is typically from a mammal including, but not limited to, primate, e.g., human; rodent, e.g., rat, mouse, hamster; cow, pig, horse, sheep, or any mammal. The nucleic acids and proteins of the invention include both naturally occurring or recombinant molecules. Exemplary human nucleotide and amino acid sequences include accession number NM_003123, NP_003114.1, J04536, AAB59540.1, and M61827.

“Membrane receptor activity,” refers to signal transduction in response to extracellular stimuli and production of second messengers such as IP3, cAMP, and Ca²⁺ via stimulation of enzymes such as phospholipase C and adenylate cyclase. Such activity can be measured by examining increases in intracellular calcium using (Offermans & Simon, *J. Biol. Chem.* 270:15175-15180 (1995)). Receptor activity can be effectively measured by recording ligand-induced changes in [Ca²⁺]_i using fluorescent Ca²⁺-indicator dyes and fluorometric imaging.

Such receptors have transmembrane, extracellular and cytoplasmic domains that can be structurally identified using methods known to those of skill in the art, such as sequence analysis programs that identify hydrophobic and hydrophilic domains (*see, e.g.,* Kyte & Doolittle, *J. Mol. Biol.* 157:105-132 (1982)). Such domains are useful for making chimeric proteins and for *in vitro* assays of the invention.

The phrase “functional effects” in the context of assays for testing compounds that modulate activity of an CD43 protein includes the determination of a parameter that is indirectly or directly under the influence of an CD43, e.g., a functional, physical, or chemical effect, such as the ability to increase or decrease mast cell degranulation and immediate hypersensitivity-type inflammatory reactions. It includes measurement of annexin, tyrosine phosphorylation, and MAP kinase activation. “Functional effects” include *in vitro*, *in vivo*, and *ex vivo* activities.

By “determining the functional effect” is meant assaying for a compound that increases or decreases a parameter that is indirectly or directly under the influence of an CD43 protein, e.g., functional, physical and chemical effects. Such functional effects can be measured by any means known to those skilled in the art, e.g., changes in spectroscopic characteristics (e.g., fluorescence, absorbance, refractive index); hydrodynamic (e.g., shape); chromatographic; or solubility properties for the protein; measuring inducible markers or transcriptional activation of the protein; measuring binding activity or binding assays, e.g. binding to antibodies; measuring changes in ligand binding activity; measuring annexin V levels, hexosaminidase release, LTC₄ release, cytokine release, MAP kinase activation, calcium mobilization, tyrosine phosphorylation of cellular proteins, cellular proliferation; measuring cell surface marker expression; measurement of changes in protein levels for CD43-associated sequences; measurement of RNA stability; phosphorylation or dephosphorylation; signal transduction, e.g., receptor-ligand interactions, second messenger concentrations (e.g., cAMP, IP3, or intracellular Ca²⁺); identification of downstream or reporter gene expression (CAT, luciferase, β-gal, GFP and the like), e.g., via

chemiluminescence, fluorescence, colorimetric reactions, antibody binding, inducible markers, and ligand binding assays.

“Inhibitors”, “activators”, and “modulators” of CD43 polynucleotide and polypeptide sequences are used to refer to activating, inhibitory, or modulating molecules identified using *in vitro* and *in vivo* assays of CD43 polynucleotide and polypeptide sequences. Inhibitors are compounds that, e.g., bind to, partially or totally block activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity or expression of CD43 proteins, e.g., antagonists. “Activators” are compounds that increase, open, activate, facilitate, enhance activation, sensitize, agonize, or up regulate CD43 protein activity. Inhibitors, activators, or modulators also include genetically modified versions of CD43 proteins, e.g., versions with altered activity, as well as naturally occurring and synthetic ligands, antagonists, agonists, antibodies, siRNA, antisense molecules, ribozymes, small chemical molecules and the like. Such assays for inhibitors and activators include, e.g., expressing CD43 protein *in vitro*, in cells, or cell membranes, applying putative modulator compounds, and then determining the functional effects on activity, as described above.

Samples or assays comprising CD43 proteins that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative protein activity value of 100%. Inhibition of CD43 is achieved when the activity value relative to the control is about 80%, preferably 50%, more preferably 25-0%. Activation of CD43 is achieved when the activity value relative to the control (untreated with activators) is 110%, more preferably 150%, more preferably 200-500% (i.e., two to five fold higher relative to the control), more preferably 1000-3000% higher.

The term “test compound” or “drug candidate” or “modulator” or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, e.g., protein, oligopeptide (e.g., from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide, oligonucleotide, etc., to be tested for the capacity to directly or indirectly modulation cellular proliferation. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, e.g., targeting compounds, rescue compounds, dimerization compounds, stabilizing compounds, addressable compounds, and

other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound (called a "lead compound") with some desirable property or activity, e.g., inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput
5 screening (HTS) methods are employed for such an analysis.

A "small organic molecule" refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 daltons and less than about 2500 daltons, preferably less than about 2000 daltons, preferably between about 100 to about 1000 daltons, more preferably between about 200 to about 500 daltons.

10 "Biological sample" include sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. Such samples include blood, sputum, tissue, cultured cells, e.g., primary cultures, explants, and transformed cells, stool, urine, etc. A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent,
15 e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 70% identity, preferably 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,
20 96%, 97%, 98%, 99%, or higher identity over a specified region (e.g., Figure 1, provided herein), when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site). Such sequences are then said to be
25 "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is
30 50-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default

program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of
5 any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison
10 can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package,
15 Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which
20 are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first
25 identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits
30 are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when:

the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN
5 program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both
10 strands.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding
15 properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly
20 encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The
25 term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses
30 any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic

acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, *et al.*, *J. Biol. Chem.* 273(52):35095-35101 (1998).

5 The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

10 The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have
15 the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical
20 compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

 Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly
25 accepted single-letter codes.

 "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to
30 essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid

variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see, e.g., Creighton, Proteins* (1984)).

Macromolecular structures such as polypeptide structures can be described in terms of various levels of organization. For a general discussion of this organization, *see, e.g., Alberts et al., Molecular Biology of the Cell* (3rd ed., 1994) and Cantor and Schimmel, *Biophysical Chemistry Part I: The Conformation of Biological Macromolecules* (1980). "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, e.g., transmembrane domains, extracellular domains, and cytoplasmic tail domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 15 to 350 amino acids long. Exemplary domains include extracellular domains, transmembrane domains, and cytoplasmic domains. Typical domains are made up of sections of lesser organization such as stretches of β -sheet and α -helices. "Tertiary structure" refers to the complete three dimensional structure of a

polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

An "siRNA" or "RNAi" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA expressed in the same cell as the gene or target gene. "siRNA" thus refers to the double stranded RNA formed by the complementary strands. The complementary portions of the siRNA that hybridize to form the double stranded molecule typically have substantial or complete identity. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA. The sequence of the siRNA can correspond to the full length target gene, or a subsequence thereof. Typically, the siRNA is at least about 15-50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferably about 20-30 base nucleotides, preferably about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

A "label" or a "detectable moiety" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region

from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization.

Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C. For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1 - 2 min. Protocols and guidelines for low and high stringency amplification reactions are provided, e.g., in Innis *et al.* (1990) *PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc. N.Y.).

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon

degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency. Additional guidelines for determining hybridization parameters are provided in numerous reference, e.g., and Current Protocols in Molecular Biology, ed. Ausubel, *et al*

"Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H - C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single

chain Fv) or those identified using phage display libraries (*see, e.g., McCafferty et al., Nature* 348:552-554 (1990))

For preparation of antibodies, e.g., recombinant, monoclonal, or polyclonal antibodies, many technique known in the art can be used (*see, e.g., Kohler & Milstein, Nature* 256:495-497 (1975); Kozbor *et al., Immunology Today* 4: 72 (1983); Cole *et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g., McCafferty et al., Nature* 348:552-554 (1990); Marks *et al., Biotechnology* 10:779-783 (1992)).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

In one embodiment, the antibody is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to Cd43 protein, polymorphic variants, alleles, orthologs, and conservatively modified variants, or splice variants, or portions thereof, can be

selected to obtain only those polyclonal antibodies that are specifically immunoreactive with CD43 proteins and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For
5 example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

10 ISOLATION OF NUCLEIC ACIDS ENCODING CD43

This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook *et al., Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular*
15 *Biology* (Ausubel *et al., eds., 1994*)).

CD43 nucleic acids, polymorphic variants, orthologs, and alleles that are substantially identical to an amino acid sequence of Figure 1 provided herein can be isolated using CD43 nucleic acid probes and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone
20 CD43 protein, polymorphic variants, orthologs, and alleles by detecting expressed homologs immunologically with antisera or purified antibodies made against human CD43 or portions thereof.

To make a cDNA library, one should choose a source that is rich in CD43 RNA. The mRNA is then made into cDNA using reverse transcriptase, ligated into a
25 recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. Methods for making and screening cDNA libraries are well known (*see, e.g., Gubler & Hoffman, Gene* 25:263-269 (1983); Sambrook *et al., supra*; Ausubel *et al., supra*).

For a genomic library, the DNA is extracted from the tissue and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The
30 fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged *in vitro*. Recombinant phage are analyzed by plaque hybridization as described in Benton & Davis, *Science* 196:180-182 (1977). Colony hybridization is carried out as generally described in Grunstein *et al., Proc. Natl. Acad. Sci. USA.,* 72:3961-3965 (1975).

An alternative method of isolating CD43 nucleic acid and its orthologs, alleles, mutants, polymorphic variants, and conservatively modified variants combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (see U.S. Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of human CD43 directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify CD43 homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of CD43 encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Gene expression of CD43 can also be analyzed by techniques known in the art, e.g., reverse transcription and amplification of mRNA, isolation of total RNA or poly A⁺ RNA, northern blotting, dot blotting, *in situ* hybridization, RNase protection, high density polynucleotide array technology, e.g., and the like.

Nucleic acids encoding CD43 protein can be used with high density oligonucleotide array technology (e.g., GeneChipTM) to identify CD43 protein, orthologs, alleles, conservatively modified variants, and polymorphic variants in this invention. In the case where the homologs being identified are linked to modulation of mast cell degranulation and immediate hypersensitivity-type inflammatory reactions, they can be used with GeneChipTM as a diagnostic tool in detecting the disease in a biological sample, *see, e.g.*, Gunthand *et al.*, *AIDS Res. Hum. Retroviruses* 14: 869-876 (1998); Kozal *et al.*, *Nat. Med.* 2:753-759 (1996); Matson *et al.*, *Anal. Biochem.* 224:110-106 (1995); Lockhart *et al.*, *Nat. Biotechnol.* 14:1675-1680 (1996); Gingeras *et al.*, *Genome Res.* 8:435-448 (1998); Hacia *et al.*, *Nucleic Acids Res.* 26:3865-3866 (1998).

The gene for CD43 is typically cloned into intermediate vectors before transformation into prokaryotic or eukaryotic cells for replication and/or expression. These intermediate vectors are typically prokaryote vectors, e.g., plasmids, or shuttle vectors.

EXPRESSION IN PROKARYOTES AND EUKARYOTES

To obtain high level expression of a cloned gene, such as those cDNAs encoding CD43, one typically subclones CD43 into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, e.g., in Sambrook *et al.*, and Ausubel *et al. supra*. Bacterial expression systems for expressing the CD43 protein are available in, e.g., *E. coli*, *Bacillus sp.*, and *Salmonella* (Palva *et al.*, *Gene* 22:229-235 (1983); Mosbach *et al.*, *Nature* 302:543-545 (1983)). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

Selection of the promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the CD43 encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operably linked to the nucleic acid sequence encoding CD43 and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. Additional elements of the cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as MBP, GST, and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, e.g., c-myc.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A⁺, pMTO10/A⁺, pMAMneo-5, baculovirus pDSVE, and any other vector
5 allowing expression of proteins under the direction of the CMV promoter, SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Expression of proteins from eukaryotic vectors can be also be regulated using
10 inducible promoters. With inducible promoters, expression levels are tied to the concentration of inducing agents, such as tetracycline or ecdysone, by the incorporation of response elements for these agents into the promoter. Generally, high level expression is obtained from inducible promoters only in the presence of the inducing agent; basal expression levels are minimal. Inducible expression vectors are often chosen if expression of
15 the protein of interest is detrimental to eukaryotic cells.

Some expression systems have markers that provide gene amplification such as thymidine kinase and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable, such as using a baculovirus vector in insect cells, with a CD43 encoding sequence under the direction of the polyhedrin
20 promoter or other strong baculovirus promoters.

The elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance
25 gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are preferably chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of CD43 protein, which are then purified
30 using standard techniques (*see, e.g., Colley et al., J. Biol. Chem.* 264:17619-17622 (1989); *Guide to Protein Purification*, in *Methods in Enzymology*, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (*see, e.g., Morrison, J. Bact.* 132:349-351 (1977); Clark-Curtiss & Curtiss, *Methods in Enzymology* 101:347-362 (Wu et al., eds, 1983)).

Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, biolistics, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g., Sambrook et al., supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing CD43.

After the expression vector is introduced into the cells, the transfected cells are cultured under conditions favoring expression of CD43, which is recovered from the culture using standard techniques identified below.

PURIFICATION OF CD43 POLYPEPTIDES

Either naturally occurring or recombinant CD43 can be purified for use in functional assays. Naturally occurring CD43 can be purified, e.g., from human tissue. Recombinant CD43 can be purified from any suitable expression system.

The CD43 protein may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (*see, e.g., Scopes, Protein Purification: Principles and Practice* (1982); U.S. Patent No. 4,673,641; Ausubel *et al., supra*; and Sambrook *et al., supra*).

A number of procedures can be employed when recombinant CD43 protein is being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to the CD43 protein. With the appropriate ligand, CD43 protein can be selectively adsorbed to a purification column and then freed from the column in a relatively pure form. The fused protein is then removed by enzymatic activity. Finally, CD43 protein could be purified using immunoaffinity columns.

A. Purification of CD43 from recombinant bacteria

Recombinant proteins are expressed by transformed bacteria in large amounts, typically after promoter induction; but expression can be constitutive. Promoter induction with IPTG is one example of an inducible promoter system. Bacteria are grown according to standard procedures in the art. Fresh or frozen bacteria cells are used for isolation of protein.

Proteins expressed in bacteria may form insoluble aggregates ("inclusion bodies"). Several protocols are suitable for purification of CD43 protein inclusion bodies. For example, purification of inclusion bodies typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells, e.g., by incubation in a
5 buffer of 50 mM TRIS/HCL pH 7.5, 50 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 0.1 mM ATP, and 1 mM PMSF. The cell suspension can be lysed using 2-3 passages through a French Press, homogenized using a Polytron (Brinkman Instruments) or sonicated on ice. Alternate methods of lysing bacteria are apparent to those of skill in the art (*see, e.g., Sambrook et al., supra; Ausubel et al., supra*).

10 If necessary, the inclusion bodies are solubilized, and the lysed cell suspension is typically centrifuged to remove unwanted insoluble matter. Proteins that formed the inclusion bodies may be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to
15 about 8 M). Some solvents which are capable of solubilizing aggregate-forming proteins, for example SDS (sodium dodecyl sulfate), 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon
20 removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. Other suitable buffers are known to those skilled in the art. Human CD43 proteins are separated from other bacterial proteins by standard separation techniques, e.g., with Ni-NTA agarose resin.

Alternatively, it is possible to purify CD43 protein from bacteria periplasm.
25 After lysis of the bacteria, when the CD43 protein exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to skill in the art. To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is
30 resuspended in ice-cold 5 mM MgSO₄ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.

B. Standard protein separation techniques for purifying CD43 proteins

Solubility fractionation

Often as an initial step, particularly if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol includes adding saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This concentration will precipitate the most hydrophobic of proteins. The precipitate is then discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, either through dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

Size differential filtration

The molecular weight of the CD43 proteins can be used to isolate it from proteins of greater and lesser size using ultrafiltration through membranes of different pore size (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

Column chromatography

The CD43 proteins can also be separated from other proteins on the basis of its size, net surface charge, hydrophobicity, and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art. It will be apparent to one

of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

IMMUNOLOGICAL DETECTION OF CD43 POLYPEPTIDES

5 In addition to the detection of CD43 genes and gene expression using nucleic acid hybridization technology, one can also use immunoassays to detect CD43 proteins of the invention. Such assays are useful for screening for modulators of CD43 regulation of mast cell degranulation and immediate hypersensitivity-type inflammatory reactions, as well as for therapeutic and diagnostic applications. Immunoassays can be used to qualitatively or
10 quantitatively analyze CD43 proteins. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

 Methods of producing polyclonal and monoclonal antibodies that react specifically with the CD43 proteins are known to those of skill in the art (*see, e.g., Coligan, Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal*
15 *Antibodies: Principles and Practice* (2d ed. 1986); and Kohler & Milstein, *Nature* 256:495-497 (1975)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g., Huse et al., Science* 246:1275-1281 (1989); Ward *et al., Nature* 341:544-546 (1989)).

20 A number of immunogens comprising portions of CD43 protein may be used to produce antibodies specifically reactive with CD43 protein. For example, recombinant CD43 protein or an antigenic fragment thereof, can be isolated as described herein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen
25 for the production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Naturally occurring protein may also be used either in pure or impure form. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays
30 to measure the protein.

 Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (e.g., BALB/C mice) or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. The animal's immune response to the immunogen preparation is monitored by

taking test bleeds and determining the titer of reactivity to the beta subunits. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see, Harlow & Lane, supra*).

5 Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see, Kohler & Milstein, Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well
10 known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof
15 by screening a DNA library from human B cells according to the general protocol outlined by Huse, *et al.*, *Science* 246:1275-1281 (1989).

 Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4
20 or greater are selected and tested for their cross reactivity against non-CD43 proteins, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μ M, preferably at least about 0.1 μ M or better, and most preferably, 0.01 μ M or better. Antibodies specific only for a particular CD43 ortholog, such as human CD43, can also be made, by
25 subtracting out other cross-reacting orthologs from a species such as a non-human mammal.

 Once the specific antibodies against CD43 protein are available, the protein can be detected by a variety of immunoassay methods. In addition, the antibody can be used therapeutically as a CD43 modulators. For a review of immunological and immunoassay procedures, *see Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover,
30 the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in *Enzyme Immunoassay* (Maggio, ed., 1980); and Harlow & Lane, *supra*.

ASSAYS FOR MODULATORS OF CD43 PROTEIN

A. Assays

Modulation of mast cell degranulation and immediate hypersensitivity-type inflammatory reactions can be assessed using a variety of *in vitro* and *in vivo* assays, as described above, and, such assays can be used to test for inhibitors and activators of CD43 protein. Such modulators of CD43 protein, which is involved in mast cell degranulation and immediate hypersensitivity-type inflammatory reactions, are useful for treating disorders related to mast cell degranulation, such as asthma and allergies. Modulators of CD43 protein are tested using either recombinant or naturally occurring, preferably human CD43.

Preferably, the CD43 protein will have a human sequence that is an ortholog of the sequence provided in Figure 1, described herein. Alternatively, the CD43 protein of the assay will be derived from a eukaryote and include an amino acid subsequence having substantial amino acid sequence identity to the sequences of Figure 1, described herein. Generally, the amino acid sequence identity will be at least 60%, preferably at least 65%, 70%, 75%, 80%, 85%, or 90%, most preferably at least 95%.

Measurement of a inhibition of mast cell degranulation phenotype on CD43 protein or cell expressing CD43 protein, either recombinant or naturally occurring, can be performed using a variety of assays, *in vitro*, *in vivo*, and *ex vivo*. A suitable physiological change that affects activity or binding can be used to assess the influence of a test compound on the polypeptide of this invention. When the functional effects are determined using intact cells or animals, one can also measure a variety of effects such as, increases or decreases in cellular proliferation, or in the case of signal transduction, hormone release, transcriptional changes to both known and uncharacterized genetic markers (e.g., northern blots), changes in cell metabolism such as cell growth or pH changes, and changes in intracellular second messengers such as cGMP.

In a preferred embodiment, CD43 modulators are assayed by screening for mast cell degranulation, as shown in Figures 3, 5, 6, and 7.

Assays to identify compounds with modulating activity can be performed *in vitro*. For example, CD43 protein is first contacted with a potential modulator and incubated for a suitable amount of time, e.g., from 0.5 to 48 hours. In one embodiment, CD43 polypeptide levels are determined *in vitro* by measuring the level of protein or mRNA. The level of CD43 protein or proteins related to CD43 signal transduction are measured using immunoassays such as western blotting, ELISA and the like with an antibody that selectively binds to the CD43 polypeptide or a fragment thereof. For measurement of mRNA,

amplification, e.g., using PCR, LCR, or hybridization assays, e.g., northern hybridization, RNase protection, dot blotting, are preferred. The level of protein or mRNA is detected using directly or indirectly labeled detection agents, e.g., fluorescently or radioactively labeled nucleic acids, radioactively or enzymatically labeled antibodies, and the like, as
5 described herein.

Alternatively, a reporter gene system can be devised using an CD43 protein promoter operably linked to a reporter gene such as chloramphenicol acetyltransferase, firefly luciferase, bacterial luciferase, β -galactosidase and alkaline phosphatase. Furthermore, the protein of interest can be used as an indirect reporter via attachment to a second reporter such
10 as green fluorescent protein (*see, e.g.,* Mistili & Spector, *Nature Biotechnology* 15:961-964 (1997)). The reporter construct is typically transfected into a cell. After treatment with a potential modulator, the amount of reporter gene transcription, translation, or activity is measured according to standard techniques known to those of skill in the art.

An activated or inhibited CD43 receptor will alter the properties of
15 downstream target enzymes, channels, and other effector proteins. Downstream consequences can be examined such as generation of diacyl glycerol and IP3 by phospholipase C, and in turn, for calcium mobilization by IP3. Receptor activation typically initiates subsequent intracellular events, e.g., increases in second messengers such as IP3, which releases intracellular stores of calcium ions. Thus, a change in cytoplasmic calcium
20 ion levels, or a change in second messenger levels such as IP3 can be used to assess receptor function. Cells expressing such receptors may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable although not necessary to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as EGTA, to distinguish
25 fluorescence response resulting from calcium release from internal stores.

Other assays can involve determining the activity of receptors which, when activated, result in a change in the level of intracellular cyclic nucleotides, e.g., cAMP or cGMP, by activating or inhibiting enzymes such as adenylate cyclase. In cases where activation of the receptor results in a decrease in cyclic nucleotide levels, it may be preferable
30 to expose the cells to agents that increase intracellular cyclic nucleotide levels, e.g., forskolin, prior to adding a receptor-activating compound to the cells in the assay.

In one embodiment, the changes in intracellular cAMP or cGMP can be measured using immunoassays. The method described in Offermanns & Simon, *J. Biol.*

Chem. 270:15175-15180 (1995) may be used to determine the level of cAMP. Also, the method described in Felley-Bosco *et al.*, *Am. J. Resp. Cell and Mol. Biol.* 11:159-164 (1994) may be used to determine the level of cGMP. Further, an assay kit for measuring cAMP and/or cGMP is described in U.S. Patent 4,115,538, herein incorporated by reference.

5 In another embodiment, phosphatidyl inositol (PI) hydrolysis can be analyzed according to U.S. Patent 5,436,128, herein incorporated by reference. Briefly, the assay involves labeling of cells with ³H-myoinositol for 48 or more hrs. The labeled cells are treated with a test compound for one hour. The treated cells are lysed and extracted in chloroform-methanol-water after which the inositol phosphates were separated by ion
10 exchange chromatography and quantified by scintillation counting. Fold stimulation is determined by calculating the ratio of cpm in the presence of agonist to cpm in the presence of buffer control. Likewise, fold inhibition is determined by calculating the ratio of cpm in the presence of antagonist to cpm in the presence of buffer control (which may or may not contain an agonist).

15

B. Modulators

The compounds tested as modulators of CD43 protein can be any small chemical compound, or a biological entity, such as a protein, e.g., an antibody, a sugar, a nucleic acid, e.g., an antisense oligonucleotide, siRNA, or a ribozyme, or a lipid.

20 Alternatively, modulators can be genetically altered versions of an CD43 protein. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by
25 automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs Switzerland) and the like.

30 In one preferred embodiment, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that

display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.*, U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al.*, *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.*, PCT Publication No. WO 91/19735), encoded peptides (*e.g.*, PCT Publication No. WO 93/20242), random bio-oligomers (*e.g.*, PCT Publication No. WO 92/00091), benzodiazepines (*e.g.*, U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.*, *Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al.*, *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al.*, *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al.*, *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al.*, *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, *all supra*), peptide nucleic acid libraries (*see, e.g.*, U.S. Patent 5,539,083), antibody libraries (*see, e.g.*, Vaughn *et al.*, *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.*, Liang *et al.*, *Science*, 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.*, benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.*, 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore,

Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.*, ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

5 In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing the CD43 protein is attached to a solid phase substrate. In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential
10 modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 96 modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay many plates per day; assay screens for up to about 6,000, 20,000, 50,000, or 100,000 or more different compounds are possible
15 using the integrated systems of the invention.

C. *Solid State and soluble high throughput assays*

In one embodiment the invention provides soluble assays using a CD43 protein, or a cell or tissue expressing an CD43 protein, either naturally occurring or
20 recombinant. In another embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the CD43 protein is attached to a solid phase substrate.

In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a
25 microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (*e.g.*, 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different compounds. It is possible to assay many plates per day; assay screens
30 for up to about 6,000, 20,000, 50,000, or more than 100,000 different compounds are possible using the integrated systems of the invention.

The protein of interest, or a cell or membrane comprising the protein of interest can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage *e.g.*, via a tag. The tag can be any of a variety of components. In general, a

molecule which binds the tag (a tag binder) is fixed to a solid support, and the tagged molecule of interest is attached to the solid support by interaction of the tag and the tag binder.

A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.) Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders; see, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).

Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs. For example, agonists and antagonists of cell membrane receptors (e.g., cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherein family, the integrin family, the selectin family, and the like; see, e.g., Pigott & Power, *The Adhesion Molecule Facts Book I* (1993). Similarly, toxins and venoms, viral epitopes, hormones (e.g., opiates, steroids, etc.), intracellular receptors (e.g. which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to persons of skill in the art. For example,

poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. See, e.g., Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen *et al.*, *J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al.*, *Science*, 251:767-777 (1991); Sheldon *et al.*, *Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al.*, *Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

CELLULAR TRANSFECTION AND GENE THERAPY

The present invention provides the nucleic acids of CD43 protein for the transfection of cells *in vitro* and *in vivo*. These nucleic acids can be inserted into any of a number of well-known vectors for the transfection of target cells and organisms as described below. The nucleic acids are transfected into cells, *ex vivo* or *in vivo*, through the interaction of the vector and the target cell. The nucleic acid, under the control of a promoter, then expresses a CD43 protein of the present invention, thereby mitigating the effects of absent, partial inactivation, or abnormal expression of an CD43 gene, particularly as it relates to mast cell degranulation and immediate hypersensitivity-type inflammatory reactions. The compositions are administered to a patient in an amount sufficient to elicit a therapeutic response in the patient. An amount adequate to accomplish this is defined as "therapeutically effective dose or amount."

Such gene therapy procedures have been used to correct acquired and inherited genetic defects, cancer, and other diseases in a number of contexts. The ability to

express artificial genes in humans facilitates the prevention and/or cure of many important human diseases, including many diseases which are not amenable to treatment by other therapies (for a review of gene therapy procedures, see Anderson, *Science* 256:808-813 (1992); Nabel & Felgner, *TIBTECH* 11:211-217 (1993); Mitani & Caskey, *TIBTECH* 11:162-166 (1993); Mulligan, *Science* 926-932 (1993); Dillon, *TIBTECH* 11:167-175 (1993); Miller, *Nature* 357:455-460 (1992); Van Brunt, *Biotechnology* 6(10):1149-1154 (1998); Vigne, *Restorative Neurology and Neuroscience* 8:35-36 (1995); Kremer & Perricaudet, *British Medical Bulletin* 51(1):31-44 (1995); Haddada *et al.*, in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al.*, *Gene Therapy* 1:13-26 (1994)).

PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (e.g., nucleic acid, protein, modulatory compounds or transduced cell), as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (see, e.g., *Remington's Pharmaceutical Sciences*, 17th ed., 1989). Administration can be in any convenient manner, e.g., by injection, oral administration, inhalation, transdermal application, or rectal administration.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be

administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of commends can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The dose will be determined by the efficacy of the particular vector employed and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, or transduced cell type in a particular patient.

In determining the effective amount of the vector to be administered in the treatment or prophylaxis of conditions owing to diminished or aberrant expression of the CD43 protein, the physician evaluates circulating plasma levels of the vector, vector toxicities, progression of the disease, and the production of anti-vector antibodies. In general, the dose equivalent of a naked nucleic acid from a vector is from about 1 μ g to 100 μ g for a typical 70 kilogram patient, and doses of vectors which include a retroviral particle are calculated to yield an equivalent amount of therapeutic nucleic acid.

For administration, compounds and transduced cells of the present invention can be administered at a rate determined by the LD-50 of the inhibitor, vector, or transduced cell type, and the side-effects of the inhibitor, vector or cell type at various concentrations, as

applied to the mass and overall health of the patient. Administration can be accomplished via single or divided doses.

EXAMPLES

5 The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Identification of CD43 as an inhibitor of IgE-receptor mediated mast cell degranulation

10 Mouse BMCC.7 cells were used with annexin V staining to identify cDNA clones that inhibited IgE-receptor mediated mast cell degranulation. BMMC cells were infected with a full-length BMMC cDNA library in a retroviral vector. The infected population was sensitized with IgE and stimulated with DNF-BSA for 30 minutes and stained with APC conjugated annexin V. The lowest 1% of annexin V-stained cells was sorted and
15 expanded. Stimulation and sorting were repeated an additional 4 times to enrich for non-responding cells. Single cell clones were isolated using RT-PCR and retested for the non-responding phenotype. The inhibitory phenotype was confirmed by phenotype transfer. Transfer of the non-responding phenotype is measured after sensitization with IgE and stimulation with anti-Ig-E and ionomycin by annexin V staining, hexosaminidase release,
20 LTC₄ release, and cytokine release (Figures 2 and 3). CD43 was shown to inhibit IgE-induced biochemical events in BMMC cells, e.g., calcium signaling, MAP kinase activation, and tyrosine phosphorylation of cellular proteins (Figure 4).

25 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

1 WE CLAIM:

- 1 1. A method for identifying a compound that modulates mast cell
2 degranulation, the method comprising the steps of:
3 (i) contacting the compound with a CD43 polypeptide, the polypeptide
4 encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid
5 encoding a polypeptide having an amino acid sequence of SEQ ID NO:2; and
6 (ii) determining the functional effect of the compound upon the CD43
7 polypeptide.
- 1 2. The method of claim 1, wherein the functional effect is measured
2 *in vitro*.
- 1 3. The method of claim 2, wherein the functional effect is a physical
2 effect.
- 1 4. The method of claim 3, wherein the functional effect is determined
2 by measuring ligand binding to the polypeptide.
- 1 5. The method of claim 2, wherein the functional effect is a chemical
2 effect.
- 1 6. The method of claim 1, wherein the polypeptide is expressed in a
2 eukaryotic host cell or cell membrane.
- 1 7. The method of claim 6, wherein the functional effect is a physical
2 effect.
- 1 8. The method of claim 7, wherein the functional effect is determined
2 by measuring ligand binding to the polypeptide.
- 1 9. The method of claim 6, wherein the functional effect is a chemical
2 or phenotypic effect.
- 1 10. The method of claim 9, wherein the chemical or phenotypic effect
2 is determined by measuring hexosaminidase release, LTC4 release, cytokine release,
3 annexin V levels, calcium mobilization, tyrosine phosphorylation of cellular proteins, or
4 MAP kinase activation after IgE sensitization and/or stimulation.

- 1 11. The method of claim 1, wherein modulation is inhibition of mast
2 cell degranulation.
- 1 12. The method of claim 6, wherein the host cell is a mast cell.
- 1 13. The method of claim 12, wherein the cancer cell is a mouse
2 BMMC cell or a JAB cell.
- 1 14. The method of claim 12, wherein the cancer cell is a transformed
2 cell line.
- 1 15. The method of claim 1, wherein the polypeptide is recombinant.
- 1 16. The method of claim 1, wherein the polypeptide is encoded by a
2 nucleic acid comprising a sequence of SEQ ID NO:1.
- 1 17. The method of claim 1, wherein the compound is an antibody.
- 1 18. The method of claim 1, wherein the compound is an antisense
2 molecule.
- 1 19. The method of claim 1, wherein the compound is an siRNA
2 molecule.
- 1 20. The method of claim 1, wherein the compound is a small organic
2 molecule.
- 1 21. The method of claim 1, wherein the compound is a peptide.
- 1 22. A method of modulating mast cell degranulation in a subject, the
2 method comprising the step of administering to the subject a therapeutically effective
3 amount of a compound identified using the method of claim 1.
- 1 23. The method of claim 22, wherein the subject is a human.
- 1 24. The method of claim 23, wherein the subject has cancer.
- 1 25. The method of claim 22, wherein the compound is an antibody.

1 26. The method of claim 22, wherein the compound is an antisense
2 molecule.

1 27. The method of claim 22, wherein the compound is an siRNA
2 molecule.

1 28. The method of claim 22, wherein the compound is a small organic
2 molecule.

1 29. The method of claim 22, wherein the compound is a peptide.

1 30. A method of modulating mast cell degranulation in a subject, the
2 method comprising the step of administering to the subject a therapeutically effective
3 amount of a CD43 polypeptide, the polypeptide encoded by a nucleic acid that hybridizes
4 under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid
5 sequence of SEQ ID NO:2.

1 31. A method of modulating mast cell degranulation in a subject, the
2 method comprising the step of administering to the subject a therapeutically effective
3 amount of a nucleic acid encoding a CD43 polypeptide, wherein the nucleic acid
4 hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an
5 amino acid sequence of SEQ ID NO:2.

FIGURE 1

SEQ ID NO:1--NM_003123

1 GCCTCGGGAG GTGGTGGAGT GACCTGGCCC CAGTGCTGCG TCCTTATCAG CCGAGCCGGT
61 CCCAGCTCTT GCTCCTGCCT GTTGCCTGG AAATGGCCAC GCTTCTCCTT CTCCTTGGGG
121 TGCTGGTGGT AAGCCCAGAC GCTCTGGGGA GCACAACAGC AGTGCAGACA CCCACCTCCG
181 GAGAGCCTTT GGTCTCTACT AGCGAGCCCC TGAGCTCAAA GATGTACACC ACTTCAATAA
241 CAAGTGACCC TAAGGCCGAC AGCACTGGGG ACCAGACCTC AGCCCTACCT CCCTCAACTT
301 CCATCAATGA GGGATCCCCT CTTTGGACTT CCATTGGTGC CAGCACTGGT TCCCCTTTAC
361 CTGAGCCAAAC AACCTACCAG GAAGTTTCCA TCAAGATGTC ATCAGTGCCC CAGGAAACCC
421 CTCATGCAAC CAGTCATCCT GCTGTTCCCA TAACAGCAAA CTCTCTAGGA TCCCACACCG
481 TGACAGGTGG AACCATAACA ACGAACTCTC CAGAAACCTC CAGTAGGACC AGTGGAGCCC
541 CTGTTACCAC GGCAGCTAGC TCTCTGGAGA CCTCCAGAGG CACCTCTGGA CCCCTCTTA
601 CCATGGCAAC TGTCTCTCTG GAGACTTCCA AAGGCACCTC TGGACCCCTT GTTACCATGG
661 CAACTGACTC TCTGGAGACC TCCACTGGGA CCACTGGACC CCCTGTTACC ATGACAAC TG
721 GCTCTCTGGA GCCCTCCAGC GGGGCCAGTG GACCCAGGT CTCTAGCGTA AAATATCTA
781 CAATGATGTC TCCAACGACC TCCACCAACG CAAGCACTGT GCCCTTCCGG AACCAGATG
841 AGAACTCACG AGGCATGCTG CCAGTGGCTG TGCTTGTGGC CCTGCTGGCG GTCATAGTCC
901 TCGTGGCTCT GCTCCTGCTG TGGCGCCGGC GGCAGAAGCG GCGGACTGGG GCCCTCGTGC
961 TGAGCAGAGG TGGCAAGCGT AACGGGGTGG TGGACGCCTG GGCTGGGCCA GCCCAGGTCC
1021 CTGAGGAGGG GGCCGTGACA GTGACCGTGG GAGGGTCCGG GGGCGACAAG GGCTCTGGGT
1081 TCCCGGATGG GGAGGGGTCT AGCCGTCGGC CCACGCTCAC CACTTTCTTT GGCAGACGGA
1141 AGTCTCGCCA GGGCTCCCTG GCGATGGAGG AGCTGAAGTC TGGGTCAGGC CCCAGCCTCA
1201 AAGGGGAGGA GGAGCCACTG GTGGCCAGTG AGGATGGGGC TGTGGACGCC CCAGCTCCTG
1261 ATGAGCCCGA AGGGGGAGAC GGGGCTGCCC CTTAAGTGTC GGTGAATAGT GAGGCTGGAG
1321 GCCGGAATCT CAGCCAGCCT CCAGCACCTT CCCTCTCACC ATCCCACTGC CCCCTCGCTC
1381 CCATGTTTCC ACCCGGCACC CTGATCCTCA CCCGAATCTC CTTTTTTTTT TTCTTTTGAG
1441 ACAGAGTTTC GCTTTGTCGC CCAGGCTGGA GTGCAATGCA CGATCTCAGT TCACTGCAAC
1501 CTCTGCCTCC TAAGTTCAGG CGATTCTCCT GCCTCAGCTT CCCGAGTAAC TGAGATTACA
1561 GGCACCCACC ACCATGCCCA GCTGCTTTTT TGTATTTTTG GTAGAGATGG GGTTCACCA
1621 TGTTGGCTAG GCTGGTCTCA AACTCCTGAC CTCAGGTGAT CTACCTGCCT CAGCCTCCCA
1681 AAGTGCTGAG ATTACAGACA TGAGCCTCCG CGCCTTGCCT CCTCACCCAC CTCTTCACTC
1741 TGAATCCTCA TGAGGCTTCT CAGCCCTGGA TTTCTGCTG CCATCCTCAC CCAGCACCCA
1801 CAACTAGCGC CTGGGCAGGG CAGGGCTGGC ACCTCTCAAC GTCTGTGGAC TGAATGAATA
1861 AACCTCCTC ATCCACCCC

SEQ ID NO:2--NP_003114.1

MATLLLLLVSPDALGSTTAVQTPSTGEPLVSTSEPLSSKMYTTSITSDPKADSTGDQTSALPPSTSINEGS
PLWTSIGASTGSPLPEPTYQEVSIMSSVPQETPHATSHPAVPITANSLGSHVTGTTITNSPETSSRTSGAP
VTTAASSLETSGTSGPPLTMATVSLETSGTSGPPVTMATDSLETSTGTTGPPVTMTTGSLEPSSGASGPQVSS
VKLSTMMSPTTSTNASTVPFRNPDENSRGMLPVAVLVALLAVIVLVALLLLWRRRQKRRTGALVLSRGGKRNQV
DAWAGPAQVPEEGAVTVTVGGSGDGKSGFPDGEGRSRRPTLTFFGRRKSRQGS LAMEELKSGSGPSLKGEERP
LVASEDGAVDAPAPDEPEGGDGAAP

1351181

Biotransfer of Degranulation Phenotype

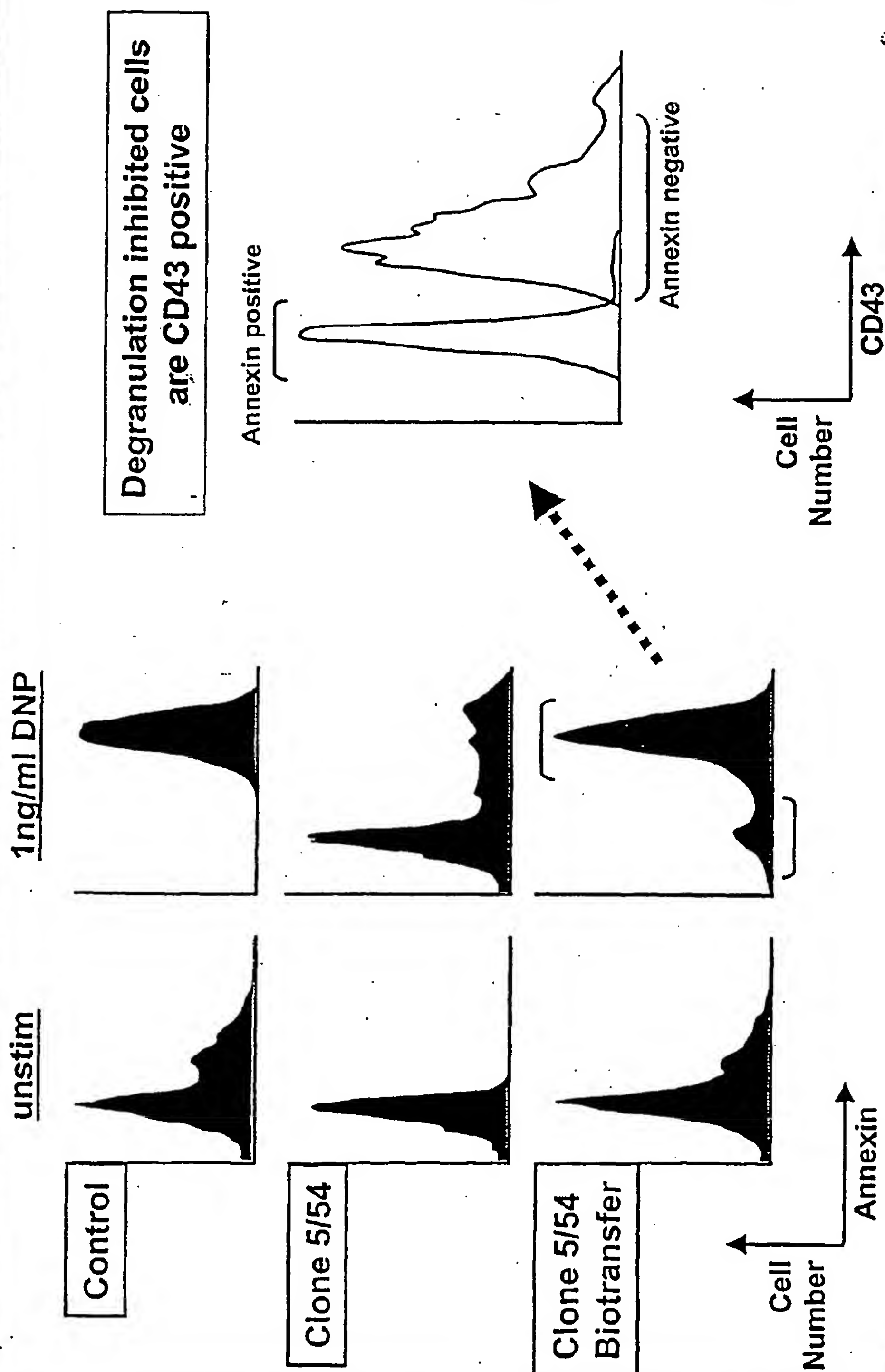


Fig. 2

CD43 Overexpression Inhibits IgE-Induced Degranulation

Full length CD43 cloned into retroviral vector and reintroduced into BMMCs. CD43+ cells sorted and subjected to analysis.

Hexosaminidase

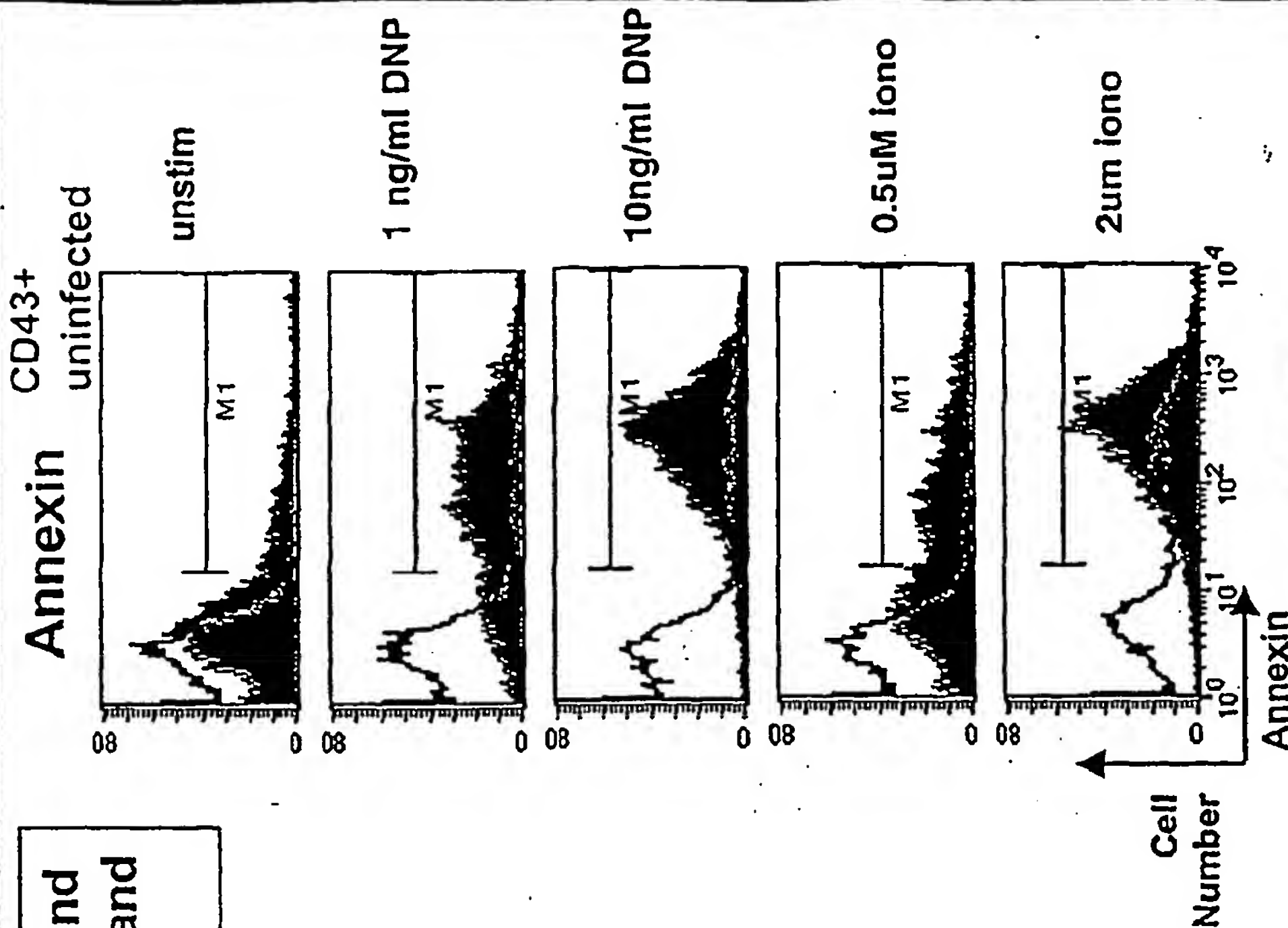
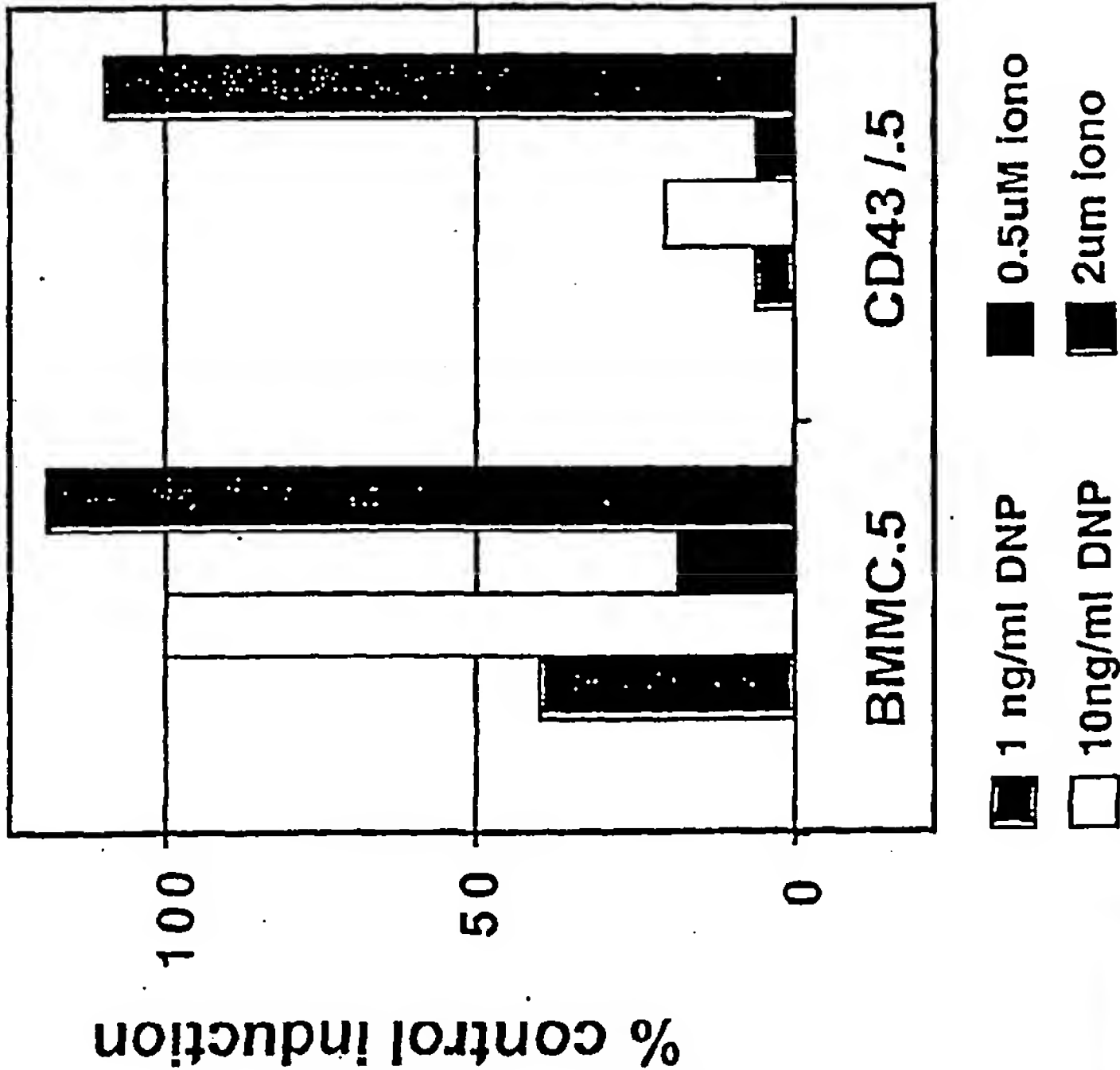
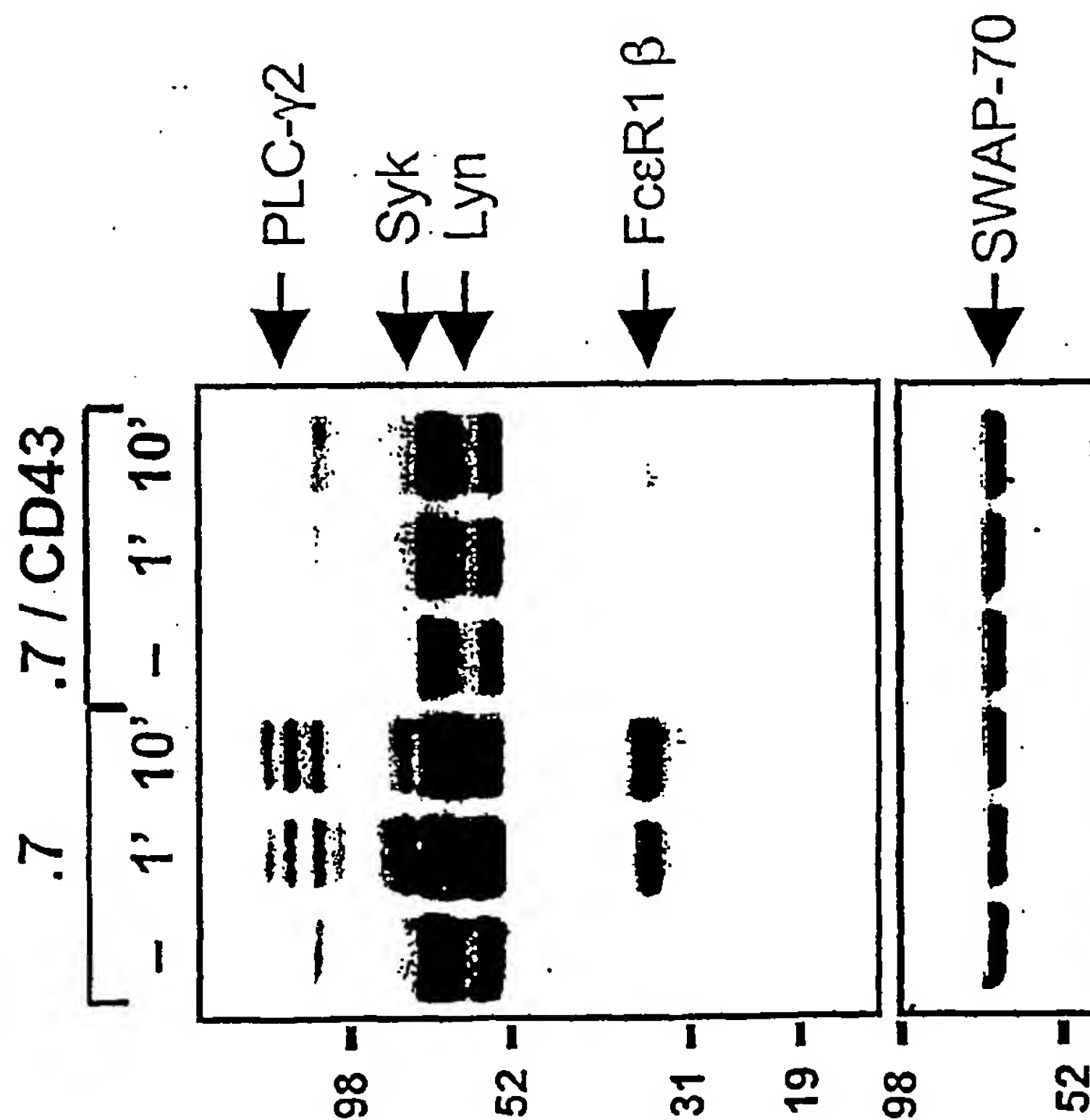


Fig. 3

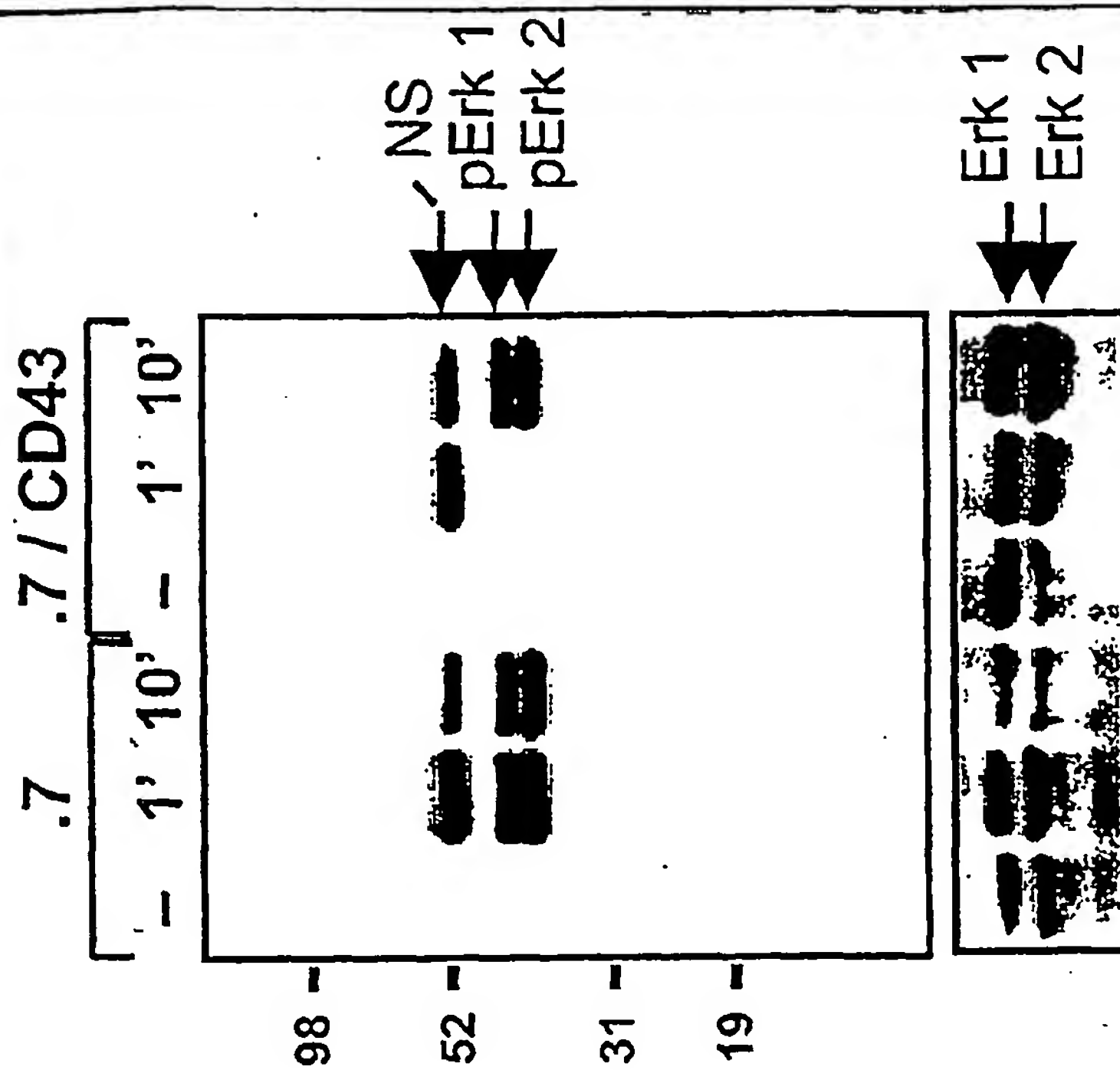
CD43 Overexpression Inhibits anti-IgE-Induced Tyrosine Phosphorylation and MAP Kinase Activation

Anti-pTyr blot of WCL



Loading control:
reprobe anti-SWAP-70

Anti-pMAPK blot of WCL



Loading control:
reprobe anti-MAPK

Fig. 4

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